

Applicant : Henry Lardy, et al.  
Application No. : 09/675,323  
Filed : September 28, 2000  
Title : Therapeutic Treatment of Androgen Driven Conditions  
5 Examiner : Elli Peselev  
TC/A.U. : 1623

Docket No. : HOLISED.063A  
Customer No. : 26551  
10 Confirmation No.: 2363

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15 **DECLARATION UNDER 37 CFR § 1.131**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

20 Dear Sir:

25 We, Henry A. Lardy and Padma Marwah declare as follows:

1. We are the inventors of the above-referenced patent application. The following statements are based on the documents identified below, our personal knowledge of the facts and results that are discussed below.

30 2. We are coauthors of the H-C. Chang, et al., publication (*Proc. Natl. Acad. Sci USA*, 96:11173-11177, 1999). We were aware of the contents of this reference before it was submitted for publication. I, Henry A. Lardy, contributed the Chang et al. reference for publication as shown at the attribution statement: "*Contributed by Henry Lardy, August 5, 1999*" on page 11173. The information in 35 this publication was therefore necessarily in my possession before the article published. The manuscript that the journal received for publication on August 5, 1999 was thus a written reduction to practice for all of the information in the article that existed on that date, which predates the article's publication on

September 28, 1999. Exhibit 1 is information the journal has provided showing that the first date the article was mailed to subscribers was September 28, 1999.

3. We selected the compounds that were described in Chang, et al. to

5 inhibit the capacity of androst-5-ene-3 $\beta$ ,17 $\beta$ -diol (referred to as 'Adiol' in Chang, et al.) to activate androgen receptor transcriptional activity. We alone selected the compounds on the basis of structural activity relationships, a full description of which has been subsequently published (Marwah, P., et al. *Bioorg. Med. Chem.*, 14:5933-5947, 2006, newly cited).

10

4. I, Henry A. Lardy, had all of the compounds, which are described in Chang, synthesized in my laboratory or commercially purchased under my direction. The attached sheet at Exhibit 2 is from a laboratory notebook describing the melting point for compound 10 (androst-5-ene-3 $\beta$ -

15 methylcarbonate-7,17-dione). This information was in my possession before I, Henry A. Lardy, submitted the Chang, et al. reference to the journal for publication. The assay described in the Chang, et al. reference was essentially the same assay that was published earlier (H. Miyamoto et al., *Proc. Natl. Acad. Sci. USA*, 95:11083-11088, 1998, of record) showing that androst-5-ene-3 $\beta$ ,17 $\beta$ -diol could activate androgen receptor transcriptional activity. I, Henry A. Lardy 20 was a coauthor of the Miyamoto et al. publication, and I was thus familiar with the assay and protocols it described before I contributed the Miyamoto et al. reference for publication as shown by the attribution statement: "*Contributed by Henry Lardy, July 29, 1998*" on page 11083.

25

5. I, Henry A. Lardy, sent the compounds, including compound 10 that were described in Chang et al. to Dr. Chang's laboratory for characterization of their activity using the previously published Miyamoto, et al. assay and the assay described in the Chang, et al. publication. The compounds were sent with the 30 molecular weights for each. Detailed structures were sent only after they had

sent us the results of the assays. The attached sheet at Exhibit 3 is from a laboratory notebook showing that compounds, including Compound 10, were sent to Dr. Chang's laboratory coded, and only we knew the results and chemical structures for individual compounds in the assay before any personnel in Dr.

5 Chang's laboratory was aware of this. In view of the facts, none of the subject matter that is now disclosed or claimed in this patent application and disclosed in Chang, et al. was or could have been invented by or derived from any of the other Chang, et al. authors.

10        6. We hereby declare that all statements made herein of my own  
knowledge are true and all statements made on information and belief are  
believed to be true; and further that these statements were made with the  
knowledge that willful false statements and the like so made are punishable by  
fine or imprisonment, or both, under Section 1001 of Title 18 of the United States  
15 Code and that such false statements may jeopardize the validity of the  
application or any patent issued thereon.

Date: February 21, 2007 By: /Henry A. Lardy/

20 Henry A. Lardy

Date: February 21, 2007 By: /Padma Marwah/

Padma Marwah

**Exhibit 1**  
**(2 sheets attached)**

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Vol. 96, Issue 20, 11173-11177, September 28, 1999

## Biochemistry

# Suppression of $\Delta^5$ -androstenediol-induced androgen receptor transactivation by selective steroids in human prostate cancer cells

Hong-Chiang Chang\*, Hiroshi Miyamoto\*, Padma Marwah†, Henry Lardy†, Shuyuan Yeh\*, Ko-En Huang\*, and Chawnshang Chang\*,†,‡

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Contributed by Henry Lardy, August 5, 1999

Our earlier report suggested that androst-5-ene- $3\beta,7\beta$ -diol ( $\Delta^5$ -androstenediol or Adiol) is a natural hormone with androgenic activity and that two potent antiandrogens, hydroxyflutamide (Eulexin) and bicalutamide (Casodex), fail to block completely the Adiol-induced androgen receptor (AR) transactivation in prostate cancer cells. Here, we report the development of a reporter assay to screen several selective steroids with anti-Adiol activity. Among 22 derivatives/metabolites of dehydroepiandrosterone, we found 4 steroids [no. 4, 1,3,5(10)-estratriene- $17\alpha$ -ethynyl- $3,17\beta$ -diol; no. 6,  $17\alpha$ -ethynyl-androstene-diol; no. 8,  $3\beta,17\beta$ -dihydroxy-

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androst-5-ene-16-one; and no. 10, 3 $\beta$ -methylcarbonate-androst-5-ene-7,17-dione] that have no androgenic activity and could also block the Adiol-induced AR transactivation in prostate cancer PC-3 cells. Interestingly, these compounds, in combination with hydroxyflutamide, further suppressed the Adiol-induced AR transactivation. Reporter assays further showed that these four anti-Adiol steroids have relatively lower glucocorticoid, progesterone, and estrogenic activity. Together, these data suggest some selective steroids might have anti-Adiol activity, which may have potential clinical application in the battle against the androgen-dependent prostate cancer growth.

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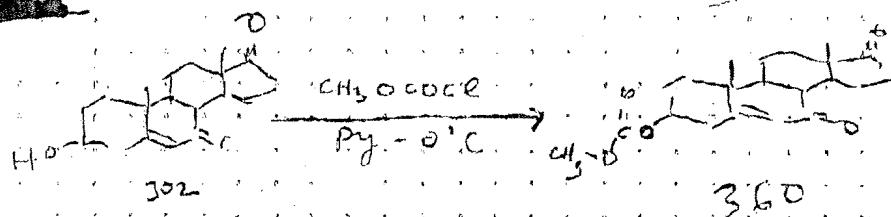
<sup>†</sup> To whom reprint requests should be addressed. E-mail: chang@pathology.rochester.edu .

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**Exhibit 2**  
**(1 sheet attached)**

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$\text{ZnO Dose} = 0.5 \text{ g} \cdot (\text{cm}^2 \text{ hr})^{1/2}$

Py. = 5 ml

$$\text{methylformate} = 0.19 \text{ gm.} = (0.002 \text{ mol})$$

(96%)

50 ml of 2000 DHEA was taken in Py. cooled to  $0 - 5^{\circ}\text{C}$ . & to it slowly methanolamine at  $0^{\circ}\text{C}$ . dropwise form. It is to be maintained at  $0 - 5^{\circ}\text{C}$  through stir at  $0^{\circ}\text{C}$ . for another 2-3 hrs. v 90% reaction is expected. in to water & cool with 10 cm<sup>2</sup> wash dry & chromatographed product eluted with 20%  $\text{CHCl}_3$ -hexane gave white solid. cryst from aq. MeOH (2:8). = 0.42 g/mg S.M. 1 gm.

89°/°  
Searched 80°  
60' N 168° E  
*Amarastris* Imp-168-70 C.T.

**Exhibit 3**  
**(1 sheet attached)**

3/2/

Compounds sent to Chawanshang &  
Androstone diol-16-one

17-estren-17 $\alpha$ -ethynyl-3 $\beta$ ,17 $\beta$ -diol —

17-estren-17 $\alpha$  " 17 $\beta$ -ol-3-one +  
17-estren-17 $\alpha$  " 17 $\beta$ -ol-3-one +  
17 $\beta$ -Androstan-17 $\alpha$  " 3 $\beta$ ,17 $\beta$ -diol —

1,3,5(10) Estren-17 $\alpha$ -ethynyl-3 $\beta$ ,17 $\beta$ -diol —  
This is a compound previously unsupplied to us.

Promisedly sent:

17-oxo-diol and 19-oxo-diol #23  
22-

3/3/

Compounds sent to Dr. Chang

3 $\beta$ -methylcarbonate of 4-oxo-DHEA

3 $\beta$ -Acetoxyandrost-17 $\beta$ TBDiMeSilyl ether-7-one

3 $\beta$ -methylandrost-5-en-17 $\beta$ ,17 $\beta$ -diol

3 $\beta$ -Methoxy-17 $\beta$ -hydroxyandrost-5-en-17-one

17-Methyl ester of marrasolice acid

3/4/

3 $\beta$ ,17 $\beta$ -dihydroxyandrost-5-en-16-one — 8

3 $\beta$ ,17 $\beta$ -dihydroxyandrost-5-en-11-one — 11

3 $\beta$ ,11 $\beta$ ,17 $\beta$ -trihydroxyandrost-5-en- — 1

? 3 $\beta$ -Acetoxy-7 $\alpha$ ,17 $\beta$ -dihydroxyandrost-5-en- — 1

3 $\beta$ ,17 $\beta$ -Androst-4-en- — 1

5(10)Estren-17 $\alpha$ -ethynyl-17 $\beta$ -ol-3-one + 2

3 $\beta$ ,12 $\beta$ -diacetoxyandrost-5-en-7-one + 1

17 $\beta$ -Hydroxyandrost-3 $\beta$ -ol- — 1